# The Impact of the Invasive Species, *Lonicera maackii*, on Soil Microbial Communities in Riparian Forests

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## INTRODUCTION

Exotic plant invasions are increasing in frequency and severity which elicits a concern for a decrease in biodiversity (Wilcove et al. 1998). These species can alter ecosystem structure, function and biodiversity by displacing native plants which shifts the floristic and faunal populations (Vitousek 1990). Examples have been documented where invasive species interrupt evolutionary pathways of native plants by competitive exclusion, niche displacement, predation, and even extinction (Mooney and Cleland 2001). Non-native plant species often alter nutrient and hydrologic cycles as well as the energy budget of native ecosystems (Mack et al. 2000). Soil microbial communities may also be affected by the invasion of non-native plant species (Kourtev et al. 2002).

The invasive shrub, Amur honeysuckle (*Lonicera maackii*) is one of the most problematic invasive species in eastern US forests (Luken and Thieret 1996). Native to Asia, *L. maackii* first arrived in North America in 1896 as an ornamental shrub and has since spread and invaded the forests of approximately 24 US states and parts of Canada (Luken and Thieret 1996). In Ohio, the species was first introduced in Oxford in 1960 and has since spread to many of the forests throughout the state (Hutchinson and Vankat 1998). The ability of this species to invade well is due to several traits such as its extended leaf phenology and ability to re-sprout after cutting (Hartman and McCarthy

2004). *Lonicera maackii* shades out native herbaceous plants and out-competes other shrubs and tree seedlings in woodland areas (Ohio DNR 2001). *Lonicera maackii* is also suspected to have allelopathic effects on native species (Hartman and McCarthy 2004).

The relationships between soil microbial diversity, soil function and soil resiliency are difficult to assess since exact microbial diversity is challenging to quantify (Nannipieri et al. 2003). Functional microbial diversity, that is the microbial activity as a whole, is primarily related to a soil's capacity to recover from stress and disturbance (Degens et al. 2001, Griffiths et al. 2000). Soils with higher microbial diversity are more resilient to physical and chemical stress than those of lower microbial diversity (Degens et al. 2001, Griffiths et al. 2000). Soil microbial communities play an integral role in nutrient cycling (Nannipieri et al. 2003). Particular interest is given to nitrogen which is found largely in organic resevoirs and is transferred to different states by microbial-mediated N-immobilization and mineralization (Nannipieri et al. 2003). In soils, various microbial populations often perform the same functions of decomposition of nutrient cycling (Kennedy and Smith 1995). Functional redundancy within soil microbial populations lessens the importance of species diversity and reinforces the interest in functional diversity (Walker 1992).

Microorganisms in the soil interact with and provide services for plants such as nutrient cycling and nitrogen fixation (Nannipier et al. 2003), mycorrhizal relationships (Barea 1991), and reduction in plant pathogens (Cook and Baker 1985). The invasion of non-native plant species may potentially alter nutrient cycles, especially when the invasive species differ from native plants in biomass quality and quantity (Ehrenfeld 2003). Many exotic plants increase nitrogen availability and alter N-fixation rates

(Ehrenfeld 2003). Potential impacts on microbial communities from invasive species often occur because non-native species differ in plant morphology, phenology, and leaf litter chemical composition compared to co-occuring native plants(Ehrenfeld 2003). Additionally, significant differences in functional soil microbial communities were detected in soils collected under two invasive and one native species in a hardwood forest (Kourtev et al. 2002).

Plants impact the functional diversity of soil microbial communities by variations among their root exudates as well as the chemical quality and the overall quantity of aboveground litter (Coleman et al. 2000, Kourtev et al. 2002). Functional diversity of soil microbial communities can be measured using the catabolic response profile (CRP) method developed by Degens and Harris (1997). The CRP method uses substrate-induced respiration responses to quantify catabolic richness and evenness as well as explain variations in substrate use in soil microbial communities. Differences in catabolic diversity have been observed using the CRP method in soils under varying land use (Degens et al. 2000), varying degrees of stress and disturbances (Degens et al. 2001), and under different species (Kourtev et al. 2002). Soils under indigenous or pasture land-use had higher catabolic evenness than soils under arable cropping regimes (Degens et al. 2000).

The objective of this study was to investigate the impact *L. maackii* has on the soil microbial communities in riparian forests of central Ohio. I hypothesized that *L. maackii* will have a significant impact on the functional diversity of soil microbial communities by decreasing catabolic evenness. To achieve this objective, I used the CRP method to determine the impact of *L. maackii* on catabolic diversity of soil microbial communities.

## METHODS

### *Study Sites*

The study was conducted in three parks along the Olentangy River in Franklin County, Ohio with *L. maackii*-dominated riparian forests. The study sites were Tuttle Park (N40º 00'45", W83º 00'57"), Kenny Park (N40º 04'51", W83º 01'45") , and Rush Run Park (N40º 04'33", W83º 01'39").

### *Soil Samples*

Samples were taken from sixteen 3m X 3m plots in each park in August of 2005. Eight plots were invaded by *L. maackii*, while eight adjacent plots were not invaded. Invaded plots contained dense *L. maackii* thickets, while non-invaded plots contained no *L. maackii* and were in close proximity to the corresponding invaded plots. The non-invaded plots served as control because they provided a baseline for functional diversity of the non-invaded soil microbial communities. Four random samples of the first 10 cm of the soil were taken from each plot. The soil for each plot was combined to reduce variability within each plot. The soil was sieved through a 2-mm mesh and stored at 4°C until analysis.

### *Catabolic Response Profile*

The CRP method was used to determine the catabolic diversity of the soil microbial communities in invaded and non-invaded plots (Degens 1998). The method

analyzes the microbial communities' short-term respiration responses to a variety of simple organic substrates. The substrates used in this assay were two amino acids, one aromatic chemical, two carbohydrates and thirteen carboxylic acids (Table 1). Substrate solutions were prepared in concentrations specific to the class of the substrate. Concentrations for amino acids and aromatic substrate solutions were 15 mM, carbohydrates were 75 mM, and carboxylic acids were 100 mM following Degens and Harris (1997). All solutions were adjusted to soil pH using simple titration before addition to the soil samples.

| Substrate | Chemical Name | Chemical Class |
|---|---|---|
| 1 | DL-mannose | carbohydrate |
| 2 | Citric acid | carboxylic acid |
| 3 | L-ascorbic acid | carboxylic acid |
| 4 | Fumaric Acid | carboxylic acid |
| 5 | L-lysine | amino acid |
| 6 | L-serine | amino acid |
| 7 | D-glucose | carbohydrate |
| 8 | Urocanic acid | aromatic chemical |
| 9 | Pantothenic acid | carboxylic acid |
| 10 | Quinic acid | carboxylic acid |
| 11 | L-tartaric acid | carboxylic acid |
| 12 | DL-malic acid | carboxylic acid |
| 13 | α-ketobutyric acid | carboxylic acid |
| 14 | Malonic acid | carboxylic acid |
| 15 | Succinic acid | carboxylic acid |
| 16 | Uric acid | carboxylic acid |
| 17 | α-ketoglutaric acid | carboxylic acid |
| 18 | D-gluconic acid | carboxylic acid |

Table 1. Substrates used in the CRP method with their corresponding number used in subsequent graphs.

Two mL of substrate solution were added to one gram of field moist soil in 25 mL serum bottles. The serum bottles were sealed tightly and incubated at room temperature for four hours. Samples were shaken three times during the incubation: immediately after substrate addition, after two hours of incubation, and after four hours of incubation, just

before the headspaces were analyzed for $CO_2$ evolution. The same procedure was used to determine the basal respiration rate using deionized water in place of a substrate solution. To analyze the headspace for evolved $CO_2$, a LI-COR infrared $CO_2$ analyzer was used. Using a syringe, 2 mL of the headspace in each bottle was collected and ran through the LI-COR analyzer.

Catabolic diversity is defined by catabolic richness and evenness, both of which are calculated using the concentration of $CO_2$ evolved in the soil sample in response to each substrate. The respiration concentrations used in all calculations subtracted the basal respiration from the measured $CO_2$ concentrations for each substrate. Richness is defined as the number of substrates used by the microbial community above the basal respiration. Evenness is defined as the variability of substrates use across the range of substrates tested, and is calculated using the Simpson-Yule index:

$$E = 1/\sum p^2_i$$

Where $p_i = r_i/\sum r_i$, the respiration response, above basal respiration, to each substrate ($r_i$) as a proportion of total respiration responses summed over all substrates ($/\sum r_i$) (Degens et al. 2000). In this study, the maximum evenness quotient is 18 (the number of substrates used), meaning all substrates were used equally.

In addition to catabolic diversity, the variations in substrate use were also analyzed. To eliminate variations in responses due to microbial biomass the catabolic response to each substrate was standardized. Standardized catabolic responses are calculated by dividing the individual response to a substrate by the average response from every plot to that substrate.

*Stastical Analysis*

The difference between the control and *L. maackii* functional evenness was evaluated using a one-way analysis of variances (ANOVA). The results from all sites were combined to find variations among all parks. The variations within each park were determined by comparing plots within each park.

The variations in substrate use are analyzed between *L. maackii* and control plots are determined by one-way ANOVAs, comparing *L. maackii* and control plots for each substrate. A principle components analysis (PCA) was performed to see separation in substrate use between *L. maackii* and control plots by comparing their standardized responses.

RESULTS

Catabolic richness was uniformly 18 (highest quotient for this assay) for all sites, meaning all substrates were catabolized above basal respiration. However, catabolic evenness varied between *L. maackii* and control plots overall and within each park (Figure 1). Tuttle and Kenny Parks exhibit no significant differences ($F_{1,14} = 2.66$, $P = 0.125$ and $F_{1,14} = 0.50$, $P = 0.493$, respectively), although *L. maackii* plots have a slightly higher evenness (Figure 1). Rush Run Park, on the other hand, shows slight significant difference ($F_{1,14} =$, $P = 0.049$) with the *L. maackii* plots having a greater evenness than the control plots. When all parks were pooled together, *L. maackii* soils express higher evenness than the control plots ($F_{1,45} = 7.52$, $P = 0.009$).

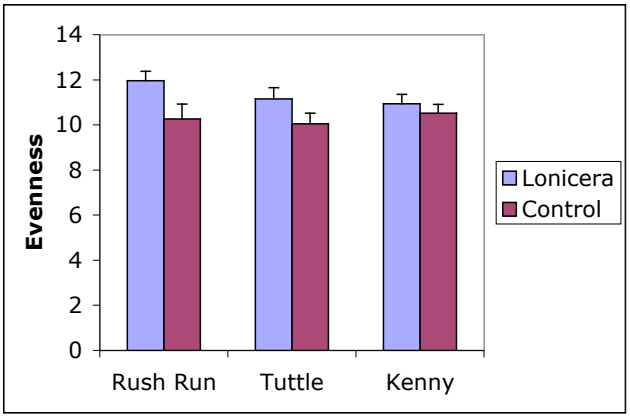


Figure 1. A comparision of microbial community functional evenness between *L. maackii* and control plots within each park.

Catabolic responses between *L. maackii* and control plots were similar for all but one substrate (Figure 2). The use of substrate 15, succinic acid, was greater under control plots than the *L. maackii* plots ($F_{1,45} = 5.83$, $P = 0.020$).

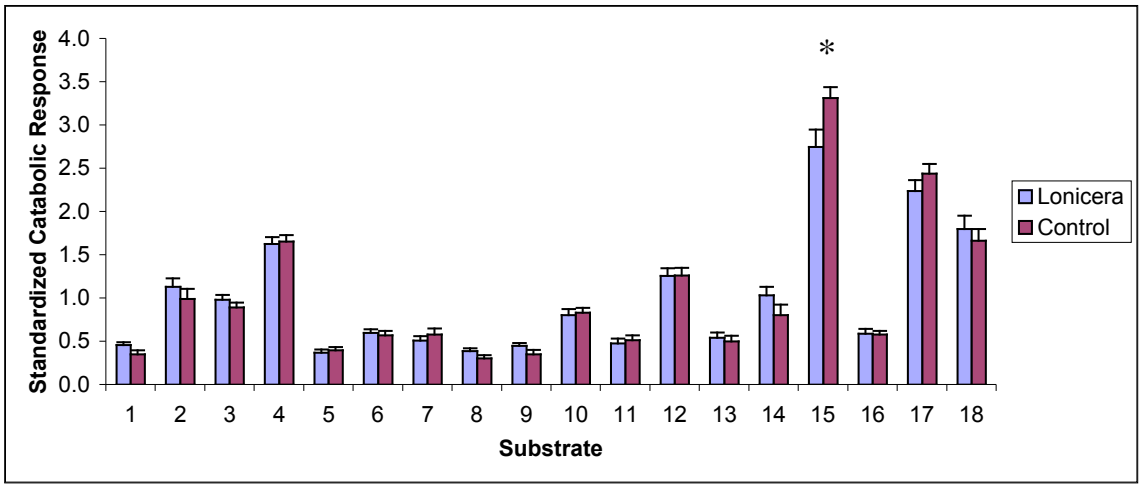


Figure 2. A comparison of respiration responses to each substrate by *L. maackii* and control plots.
* Indicates significant difference in utilization of substrate

The principle components analysis of substrate use between *L. maackii* and control plots indicates no separation between *L. maackii* and control (Figure 3). However, all plots in Kenny Park were isolated to the bottom right quadrant, whereas Tuttle Park and Rush Run Park do not separate from each other.

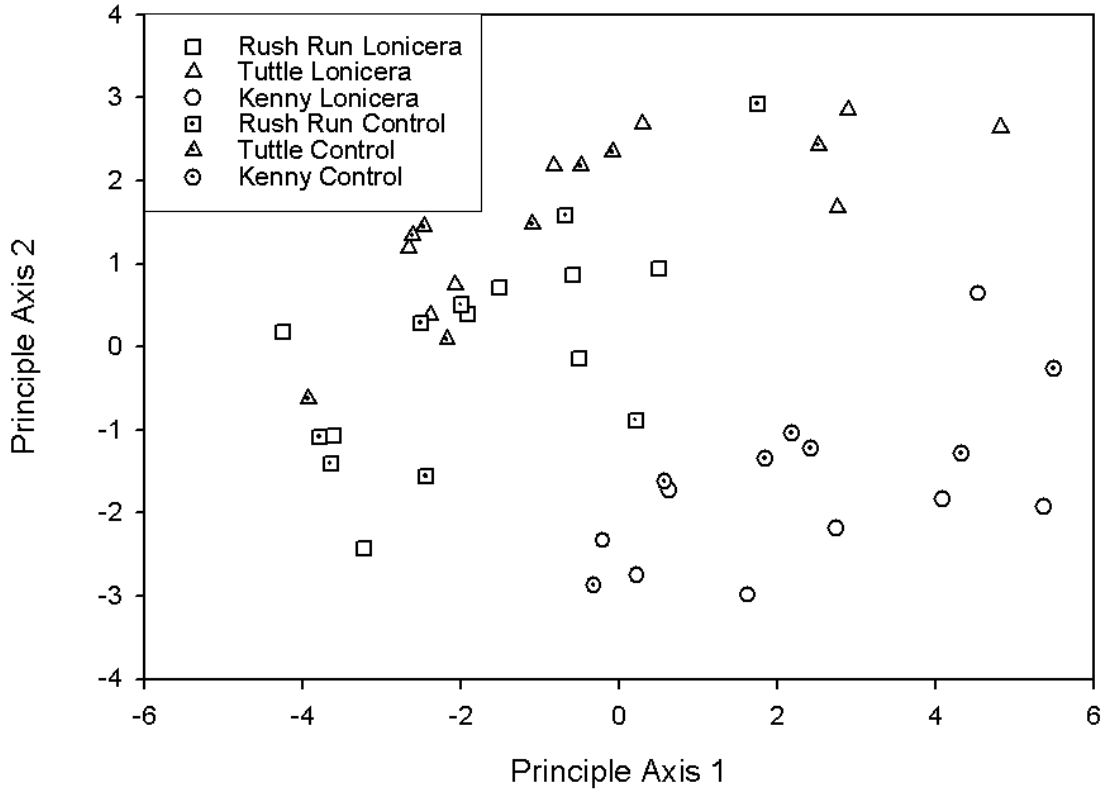


Figure 3. PCA of substrate use between each park, indicated by each symbol, and *Lonicera* and Control plots, indicated by a dot (control) or unmarked (*L. maackii*).

| Substrate | Chemical Name | Chemical Class | F value | P value |
|---|---|---|---|---|
| 4 | Fumaric Acid | carboxylic acid | 21.64 | 0.017 |
| 5 | L-lysine | amino acid | 48.07 | 0.005 |
| 6 | L-serine | amino acid | 27.96 | 0.011 |
| 7 | D-glucose | carbohydrate | 44.10 | 0.006 |
| 18 | D-gluconic acid | carboxylic acid | 30.04 | 0.010 |

Table 2. Substrates used differently by Kenny Park than Rush Run and Tuttle Park. These substrates drive the separation of Kenny from the other two parks in the PCA. Differences determined using a one-way ANOVA for substrate use between parks (F and P values from ANOVA given).

## DISCUSSION

Soil microbial functional diversity serves as an indicator for a soil's resilience to

stress and disturbance (Degens et al 2001). In this study, I hypothesized that *L. maackii* would decrease the functional diversity of the soil microbial communities in riparian forests. On the contrary, functional evenness was higher in *L. maackii* plots compared to non-invaded control plots when all parks were combined. Invasive plants often increase net primary productivity and biomass, as well as produce litter with higher decompostion rates (Ehrenfeld 2003) which may cause microbial populations to flourish. Environmental conditions in each park may have an impact on soil microbial functional diversity in addition to the plant species present. Differences in microbial functional diversity in soils under a range of stress and disturbances as well as land uses (Degens et al. 2000, Degens et al. 2001). The parks may vary in vegetation community structure, human traffic, and influence from surrounding urban areas. Soil types between parks are dissilimar which may provide insight into the differences between parks.

An analysis of variations in substrate use between *L. maackii* and control plots showed no significant differences with the exception of succinic acid. Control plots demonstrate higher catabolization of this substrate than *L. maackii* plots. Succinic acid, like most of the substrates, is commonly found in plant and animal tissue (Andersen et al. 1988). Further research should be done to determine why soil microbial communities under natural forest vegetation utilize succinic acid more than the microbial communities found under *L. maackii*.

The principle component analysis provided another frame of reference to analyze substrate use between plots and parks. The PCA indicated no separation of substrate use between plots; in fact they almost completely overlap each other. However, when analyzed by park, Kenny Park separates to one quadrant of the PCA, indicating that the

soil microbes in this park uses the substrates differently than the other parks. The variation of Kenny Park may be due to external factors, such as the degree of human and urban influence on the park, the vegetation structure, or soil properties.

Overall, there is no evidence that *L. maackii* has a substantial impact on soil microbial communities in riparian forests. Significant variations of soil microbial diversity has been detected when two invasive species were compared to a co-occurring native species (Kourtev et al. 2002). Soil samples were taken from the bulk soil and rhizosphere under each invasive and native species and variations in microbial diversity were detected (Kourtev et al. 2002). Significant differences in substrate use and catabolic evenness were detected in both rhizosphere and bulk soil samples, although rhizosphere soils elicited stronger results (Kourtev et al. 2002). Possible reasons for low variations between *L. maackii* and control plots are that the control was not taken from a uniform species. Instead the control was taken in three by three meter areas that simply were not invaded by *L. maackii*. If plots were taken from a native shrub, then compared to the *L. maackii* there could potentially be stronger results. In addition, soil samples were taken from the bulk soil, opposed to the rhizosphere which has more microbial activity and could have produced stronger results. Finally, it is conceivable that *L. maackii* does not significantly impact the soil microbial communities in riparian forests. When soils from 20 indigenous forest species were analyzed with the same technique, the microbes demonstrated similar respiration responses to the range of substrates tested, suggesting similarities in functional microbial diversity across a wide range of forest species (Stevenson et al. 2004). Soils in forests may be influenced by many plant species and the presence of one may not directly affect the microbial communities in the soil. Further

research is needed to determine if there is a greater variation found from specific native shrubs to the invasive species, *L. maackii* and if a greater difference is found in the rhizosphere of these plants.

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